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DOI:

[10.1016/j.bbi.2011.03.008](https://doi.org/10.1016/j.bbi.2011.03.008)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Suliman Khanfer, R, Lord, J & Phillips, A 2011, 'Neutrophil function and cortisol: DHEAS ratio in bereaved older adults', *Brain, Behaviour, and Immunity*, vol. 25, no. 6, pp. 1182-1186. <https://doi.org/10.1016/j.bbi.2011.03.008>

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Neutrophil function and cortisol:DHEAS ratio in bereaved older adults

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Abstract

Bereavement is a common life event for older adults and is associated with increased risk of morbidity and mortality, though the underlying reasons for this link are poorly understood. Although physical and emotional stressors and ageing are known to suppress immunity, few studies have explored the impact of bereavement upon immunity in the older population. We therefore hypothesised that the emotional stress of bereavement would suppress immune function, specifically neutrophil bactericidal activity, in older adults. A between-subjects design was used to examine the effect of recent bereavement (<2 months) on neutrophil function in elders. Participants were 24 bereaved and 24 age- and sex- matched non-bereaved controls all aged 65+ years. Neutrophil phagocytosis of *Escherichia coli* (*E. coli*) and stimulated superoxide production were assessed. Cortisol and dehydroepiandrosterone-sulphate (DHEAS) levels were determined in serum to assess potential mechanisms. Depressive and anxiety symptoms were measured by questionnaire. Neutrophil superoxide production was significantly reduced among the bereaved when challenged with *E. coli* ($p = 0.05$), or phorbol 12-myristate 13-acetate ($p = 0.009$). Further, the bereaved group had a significantly higher cortisol:DHEAS ratio compared to controls ($p = 0.03$). There was no difference in neutrophil phagocytosis between the two groups. The psychological questionnaire results showed that the bereaved had significantly greater depressive and anxiety symptoms than the non-bereaved. The emotional stress of bereavement is associated with suppressed neutrophil superoxide production and with a raised cortisol:DHEAS ratio. The stress of bereavement exaggerates the age-related decline in HPA axis and combines with immune ageing to further suppress immune function, which may help to explain increased risk of infection in bereaved older adults.

1. Introduction

Bereavement is considered to be one of the most stressful life events, becoming more frequent as we age, yet the impact upon health is under researched. This is due in large part to the difficulty of accessing this vulnerable group. What is clear is that bereavement, particularly in older adults, is associated with higher risk of morbidity and mortality (Biondi and Picardi, 1996; Clayton,

1990; Manor and Eisenbach, 2003; Stroebe et al., 2007). To try to understand the basis of this association a small number of studies have explored the impact of bereavement upon immunity (Calabrese et al., 1987; Gerra et al., 2003; Goodkin et al., 1996). Bereaved women showed reduced Natural Killer cell activity and increased plasma cortisol levels compared to non-bereaved controls (Irwin et al., 1987). Bereaved parents showed significantly decreased numbers of T-regulatory cells, and significantly increased T-helper cells compared to their matched controls and this effect persisted over 8 months (Spratt and Denney, 1991). More recently, it was found that HIV patients who experienced maladaptive grief following bereavement showed more rapid losses of CD4 T-cells over time, even when statistically adjusting for age, health status, and use of antiretroviral medications (Goforth et al., 2009).

However, there are very few studies that have examined the impact of bereavement upon immunity in older adults despite the fact that the chances of experiencing a significant bereavement increase with age. This is an important issue as it is now well established that ageing itself results in a decline in immune status, termed immunosenescence (Shaw et al., 2010), with significant consequences for susceptibility to infection. These age-related changes include decreased neutrophil function, with older adults showing a reduction in neutrophil phagocytic ability and superoxide production (Butcher et al., 2001; Hajishengallis, 2010; Lord et al., 2001; Wessels et al., 2010). As stress also suppresses immune function (Segerstrom and Miller, 2004), we propose that stress and ageing combined will have an additive and deleterious effect upon immunity, with significant health consequences for the bereaved older adult.

In support of this notion, we have previously shown that older adults who have suffered bereavement in the past 12 months had a poorer antibody response to the annual influenza vaccination in comparison to non-bereaved older adults (Phillips et al., 2006). We have also shown that a significant physical stress, hip fracture, can worsen neutrophil bactericidal ability in older adults, which was associated with increased susceptibility to infection following surgery (Butcher et al., 2003). One suggested mechanism for this effect was the increased cortisol:DHEAS ratio observed in older adults as a result of adrenopause (Orentreich et al., 1984), which is then exaggerated in older patients by the stress of hip fracture (Butcher et al., 2005). Both cortisol and DHEAS are outputs of the hypothalamic pituitary adrenal (HPA) axis. It is known that cortisol has mainly immunosuppressive actions while DHEAS is immune enhancing. Further, previous research indicates that with ageing there is imbalance between the two hormones levels, due to reducing DHEAS levels from age 30 years onwards, and relatively stable cortisol levels (Phillips et al., 2007). This reported increased cortisol:DHEAS ratio is thought to be a contributing factor to the process of immunosenescence (Buford and Willoughby, 2008). Importantly, we showed very recently that neutrophil function was enhanced by DHEAS in vitro (Radford et al., 2010), and that this steroid can overcome the suppressive effects of cortisol on neutrophil function (Butcher et al., 2003; Radford et al., 2010).

As yet, it is unknown whether a psychological stressor such as bereavement can influence neutrophil function. Such an association might underlie the link between bereavement and morbidity and mortality, particularly from pneumonia, in older adults. Further, the biological mechanisms by which bereavement modulates immune function are unknown. Consequently, the present study examined the association between bereavement and neutrophil function in healthy

older adults by comparing those who had recently suffered bereavement to an age- and sex-matched non-bereaved control group. Cortisol and DHEAS levels were also measured to examine whether any group differences in neutrophil function were associated with either hormone or differences in the cortisol:DHEAS ratio.

2. Methods

2.1. Participants

Forty-eight healthy older adults (32 females) were recruited from St. Mary's Hospice and from the community population in Birmingham, UK, between 2008 and 2010. Their mean (SD) age and body mass index (BMI) was 72.7 (5.30) years and 25.7 (3.56) kg/m². All but one Asian participant reported their ethnicity as white. The majority were non-smokers (93.7%), and 77% were classified as from a manual occupational household. Individuals were excluded if they had an acute infection or suffered from an immune disorder. Twenty-four of the participants had suffered a significant bereavement in the past 2 months.

2.2. Study design and procedure

The study was a between-subjects design. Blood samples were taken in the morning (09.00–10.00) within 2 months of bereavement of a close family member or friend. On the same day an age- and sex-matched non-bereaved control participant was recruited to provide a blood sample. Assays for neutrophil function (phagocytosis and superoxide production) were performed on the same day. Serum was frozen for later assay of the levels of the stress hormones cortisol and DHEAS. Following blood sampling, all participants completed a questionnaire pack. The study was approved by the South Birmingham NHS Local Research Ethics Committee and all participants provided their written informed consent.

2.3. Blood sampling and immune assays

Blood was collected into a heparin containing tube for analysis of neutrophil function on the same day and in another anti-coagulant free tube for later hormone analysis. Serum from this tube was frozen at 20 °C until assays for cortisol and DHEAS were performed by ELISA (IBL International, Hamburg, Germany). Neutrophil phagocytic ability and efficiency were assayed in whole blood using a commercial kit (Phagotest, Orpegen Pharma GmbH, Heidelberg, Germany) which measures

uptake of fluorescein iso-thiocyanate-(FITC) labelled opsonized E. coli. Flow cytometry (Dako Cyan, Carpinteria, California) was used to gate on neutrophils and determine the mean fluorescence intensity (MFI) corresponding to the amount of bacteria engulfed per cell and the number of cells with phagocytic function. These values were used to define the phagocytic index (% phagocytic cells MFI). Production of reactive oxidant species following stimulation with either opsonised E. coli or 12-phorbol, 13-myristate (PMA) was measured using a commercial lucigenin-based kit and according to the manufacturer's instructions (Phagoburst, Orpegen Pharma). The formation of reactive oxidants was monitored by the oxidation of the substrate dihydrorhodamine 123 to rhodamine, detected by flow cytometry.

2.4. Questionnaires

Standard socio-demographic and health behaviour questions including date of birth, sex, ethnicity, and occupational classification were asked as part of a questionnaire pack presented during the testing session. Health behaviours over the year preceding entry to the study were assessed using questions adapted from the Whitehall II study (Marmot et al., 1991), regarding smoking, alcohol consumption, exercise, sleep quantity, and diet.

Psychological morbidity was measured using the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983). The scale contains 14 four-point items, from 0 (not present) to 3 (considerable), with seven assessing largely the anhedonic rather the somatic aspects of depression (e.g., "I have lost interest in my appearance") and seven assessing anxiety (e.g., "I feel tense or wound up"). The HADS has good concurrent validity and performs well as a psychiatric screening device (Herrmann, 1997; Snaith,

2003). For the present sample, Cronbach's alpha was 0.90 for depressive symptoms and 0.88 for anxiety symptoms.

2.5. Statistical analyses

The differences between the bereaved and non-bereaved groups, in the demographic, health behaviours, and psychological variables were analysed using t-test or chi-square. Neutrophil function and hormone levels were skewed, so were subject to log transformation. Univariate analysis of variance (ANOVA) was used to assess the differences in neutrophil function and hormone levels between the bereaved and control groups. The cortisol:DHEAS ratio was calculated and compared between the groups using ANOVA. Effect sizes are reported in terms of g^2 . Correlations were then used to ascertain whether the cortisol:DHEAS ratio or any other significant demographic or psychological variables were potential mechanisms underlying the association between bereavement and neutrophil function.

3. Results

3.1. Descriptive statistics

Table 1 shows the descriptive statistics of demographic information and questionnaire scores for the two groups. The bereaved

[Insert Table 1 and Figures 1 & 2 about here]

group had significantly greater depressive symptoms, $t(45) = 2.662$, $p = 0.01$, and anxiety symptoms, $t(44) = 3.406$, $p = 0.001$, than the non-bereaved controls. None of the participants were taking medication that has been described as modifying immune function.

3.2. Neutrophil function

Bereaved participants showed significantly lower superoxide production in response to *E. coli*, $F(1,45) = 4.07$, $p = 0.05$, $g^2 = 0.083$, and PMA, $F(1,46) = 7.46$, $p = 0.009$, $g^2 = 0.139$, as illustrated in Fig. 1. The raw means (SD) for superoxide production against *E. coli* and PMA respectively for the bereaved versus non-bereaved were 141.3 (86.16) versus 201.6 (162.57), and 237.3 (153.15) versus 341.2 (158.70). However, for neutrophil phagocytosis of *E. coli*, there was no significant group difference between the bereaved and non-bereaved, $F(1,45) = 1.89$, $p = 0.18$, $g^2 = 0.040$, although the bereaved showed somewhat lower phagocytic ability; mean (SD) of raw values = 185.0 (74.18) versus 259.2 (172.74).

3.3. Serum cortisol and DHEAS

Analysis revealed a significant difference in the cortisol:DHEAS ratio, $F(1,46) = 5.17$, $p = 0.03$, $g^2 = 0.101$, such that, as illustrated in Fig. 2, there was higher cortisol:DHEAS ratio among the bereaved group than the non-bereaved (raw mean (SD) = 332.4 (442.80) versus 150.5 (102.8)). The differences in cortisol or DHEAS alone did not reach statistical significance, although they indicated higher cortisol, mean (SD) = 120.0 (65.21) versus 87.8 (44.36); $p = 0.051$ and lower DHEAS 0.69 (0.57) versus 0.79 (0.46); $p = 0.49$ in the bereaved group. Correlations revealed that the cortisol:DHEAS ratio, or the individual hormone levels were not significantly associated with the magnitude of superoxide production to *E. coli* or PMA. Neither were depressive symptoms nor anxiety scores related to superoxide production. However, depressive symptoms ($p = 0.02$) and

[Insert Figure 3 about here]

anxiety symptoms ($p < 0.001$) scores were significantly positively associated with the cortisol:DHEAS ratio, such that individuals with a higher ratio exhibited greater depressive and anxiety symptoms (see Fig. 3).

4. Discussion

In the present study bereaved older adults showed reduced neutrophil function, specifically lower neutrophil superoxide production, compared to the control group when neutrophils were challenged with either the physiologically relevant stimulus *E. coli* bacteria or the potent protein kinase C activator PMA. The bereaved group also had a significantly higher cortisol:DHEAS ratio compared to the controls although this did not relate to neutrophil function. For neutrophil phagocytosis, there was lower phagocytic ability among the bereaved group, but the difference did not meet statistical significance. The psychological questionnaire scores showed that the bereaved had significantly greater depressive and anxiety symptoms than the non-bereaved controls, and that this related to having a greater cortisol:DHEAS ratio.

These findings are consistent with the previous research into bereavement effects on other parameters of the immune system such as T-cell numbers and NK cell activity (Goforth et al., 2009; Goodkin et al., 1996; Irwin et al., 1987; Spratt and Denney, 1991), and are unique as far as we are aware in considering bereavement in the context of an aged immune system. Moreover, this is the first study to examine the effect of recent bereavement on neutrophil function in older adults, but the data have strong parallels with our previous work showing that neutrophil function is reduced as a result of physical stress (hip fracture) in seniors (Butcher et al., 2003). Tsukamoto et al. (2002) have also shown that older adults who have fewer stress coping factors, such as hobbies and close links with friends and family, had lower neutrophil superoxide production compared to those with greater numbers of these factors. Similarly, a study on the effect of stress and depression on neutrophil function among children, has shown that neutrophil superoxide production but not neutrophil phagocytosis was reduced among children with major depressive disorder (MDD) compared to controls (Bartlett et al., 1997). However, it should be acknowledged that the nature of the psychological distress and the population sampled in that study differed from the present research, and depressive symptoms did not relate to neutrophil function in our data presented here. It is possible that major depressive disorder but not depressive symptomatology can influence neutrophil function, although further research would be needed to test this possibility.

The psychological stress of bereavement is considered relatively chronic compared to the acute psychological stressors lasting for minutes or hours. The present results support previous findings of an association between chronic stress and immune dysfunction among older adults (Gouin et al., 2008). For example, the chronic stress of caregiving for a spouse with dementia was found to relate

to a lower influenza vaccination response among elderly caregivers (Kiecolt-Glaser et al., 1996). Our present findings of greater depressive and anxiety symptoms among the bereaved is also supported by previous research showing that older adults who reported higher anxiety and depression symptoms had delayed wound healing compared to those with fewer symptoms (Cole-King and Harding, 2001). In our previous study on the effects of hip fracture on neutrophil function in older adults, (Butcher et al., 2003) we observed that the cortisol:DHEAS ratio was increased compared to the healthy controls. Cortisol is known to suppress neutrophil superoxide generation (Bekesi et al., 2000), whereas DHEAS enhances this function (Radford et al., 2010). However, as this ratio was significantly affected by bereavement but not correlated with neutrophil function in the present study, the mechanism driving the observed neutrophil function changes remains unknown. It is possible that the altered cortisol:DHEAS ratio seen in this study contributes to the higher depressive and anxiety symptoms among the bereaved, given the observed significant positive correlations between the depressive and anxiety symptoms scores and the cortisol:DHEAS ratio and the reported opposing effects of cortisol and DHEAS upon mood (Duval et al., 2006; Morsink et al., 2007).

The present study has some limitations. First, the sample size was small. However, it should be noted that recruiting bereaved older participants with no co-morbidity and within two months of their bereavement is exceptionally difficult, and this sample size took more than 2 years to be achieved. Also the sample size is comparable to those in previous studies focused on bereavement and immunity (Gerra et al., 2003; Kemeny et al., 1995; Lindstrom, 1997; Spratt and Denney, 1991).

In conclusion the current study provides novel preliminary evidence that bereavement is associated with reduced neutrophil bactericidal function among older adults. Clearly the bereaved group showed lower neutrophil superoxide production to PMA and *E. coli*, and also exhibited a higher cortisol:DHEAS ratio compared to non-bereaved controls. The current study is the first study we know of to examine the effect of bereavement on neutrophil function, particularly among older adults. Indeed, given the very small number of studies that focused on bereavement and innate immunity, the novel findings could help to explain the underlying mechanisms of the higher morbidity and mortality and poorer psychological state among bereaved older adults. In addition, a similar study in younger adults would be valuable in determining whether or not neutrophil effects extend to younger bereaved individuals or are specific to those with coexisting immunosenescence. Considering the decline in neutrophil function with ageing and the higher associated morbidity, we believe the current results are valuable and indicate a need to minimise factors that will further compromise the immune system in bereaved older adults, such as social isolation.

Acknowledgments

This project was funded by the Dunhill Medical Trust and the European Commission PITN-GA-2009-238665. The authors would like to thank St. Mary's Hospice, Birmingham, UK also for help with recruitment.

Appendices

Variable	Total participants	Bereaved	Non-bereaved	p
	Mean (SD)			
Age	72.7 (53.00)	72.0 (53.80)	73.3 (5.25)	.39
HADS depression	3.9 (4.31)	5.5 (5.26)	2.4 (2.37)	.01
HADS anxiety	6.2 (4.47)	8.2 (4.80)	4.2 (3.02)	.001
BMI	25.7 (3.56)	25.8 (3.74)	25.7 (3.46)	.87
Alcohol units intake per week	1.2 (0.94)	1.5 (0.99)	1.0 (0.83)	.06
Exercise score	6.9 (6.98)	5.6 (4.68)	8.4 (8.57)	.17
	N (%)			
Current smoker	3 (6.3)	2 (8.3)	1 (4.2)	.55
Takes vitamins	23 (43.7)	11 (45.8)	10 (41.7)	.67
Sleep (<6 h per night)	9 (18.8)	3 (12.5)	6 (25.0)	.27
Occupation – manual	37 (77.1)	18 (75.0)	19 (79.2)	.94
Ethnicity – white	47 (97.9)	24 (100.0)	23 (95.8)	.31

Table 1

Descriptive statistics for participant demographics and questionnaire scores.

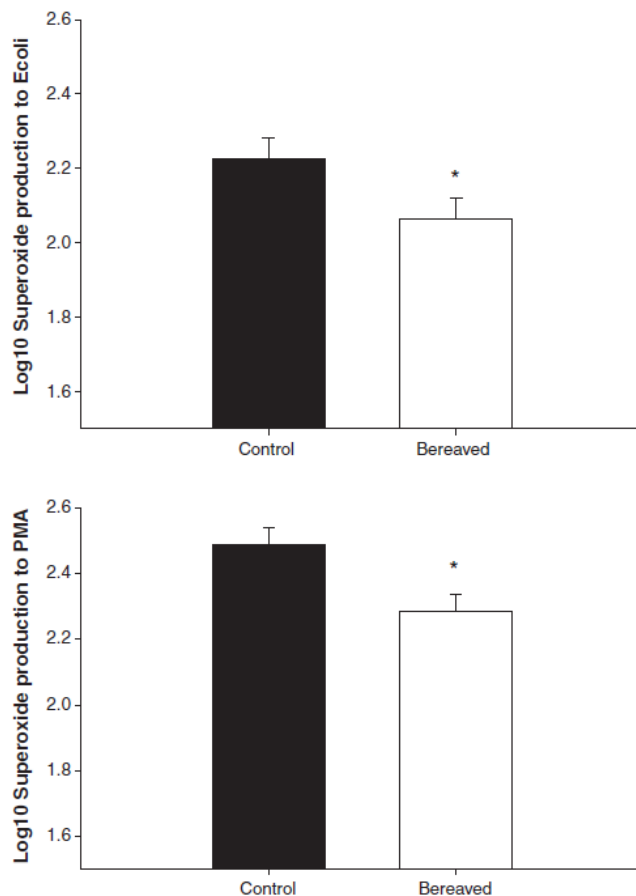


Fig. 1. Neutrophil superoxide production on stimulation with *Escherichia coli* (top), or with PMA (bottom), between the bereaved and non bereaved groups, error bars are standard error of the mean.

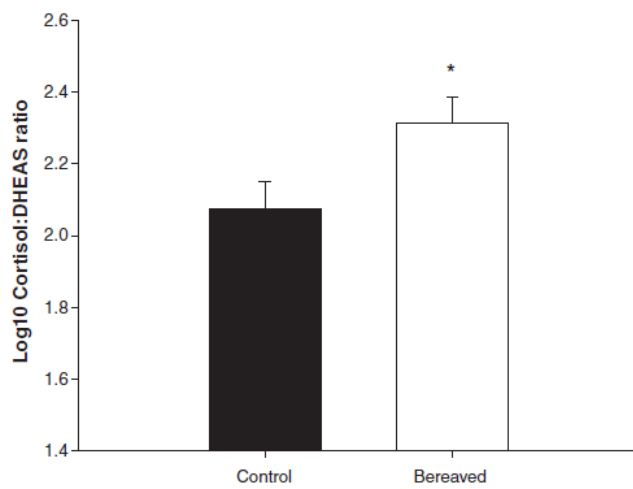


Fig. 2. The serum cortisol to DHEAS ratio compared between the bereaved and non bereaved groups, error bars are standard error of the mean.

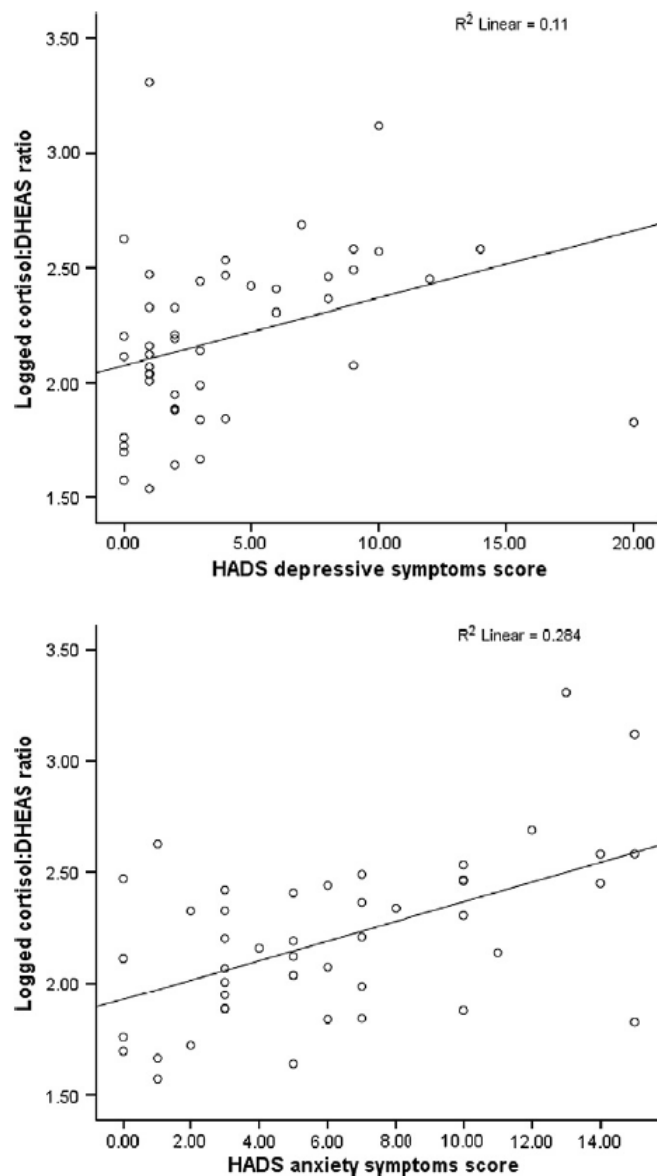


Fig. 3. Correlations between HADS scores and the cortisol:DHEAS ratio.

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